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Development of a Test System To Evaluate Procedures for Decontamination of Respirators Containing Viral Droplets[▽]

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The aim of this study was to develop a test system to evaluate the effectiveness of procedures for decontamination of respirators contaminated with viral droplets. MS2 coliphage was used as a surrogate for pathogenic viruses. A viral droplet test system was constructed, and the size distribution of viral droplets loaded directly onto respirators was characterized using an aerodynamic particle sizer. The sizes ranged from 0.5 to 15 μm, and the sizes of the majority of the droplets were the range from 0.74 to 3.5 μm. The results also showed that the droplet test system generated similar droplet concentrations (particle counts) at different respirator locations. The test system was validated by studying the relative efficiencies of decontamination of sodium hypochlorite (bleach) and UV irradiation with droplets containing MS2 virus on filtering facepiece respirators. It was hypothesized that more potent decontamination treatments would result in corresponding larger decreases in the number of viable viruses recovered from the respirators. Sodium hypochlorite doses of 2.75 to 5.50 mg/liter with a 10-min decontamination period resulted in approximately 3- to 4-log reductions in the level of MS2 coliphage. When higher sodium hypochlorite doses (≥8.25 mg/liter) were used with the same contact time that was used for the dilute solutions containing 2.75 to 5.50 mg/liter, all MS2 was inactivated. For UV decontamination at a wavelength of 254 nm, an approximately 3-log reduction in the level of MS2 virus was achieved with dose of 4.32 J/cm^2 (3 h of contact time with a UV intensity of 0.4 mW/cm^2), while with higher doses of UV irradiation (≥7.20 J/cm^2; UV intensity, 0.4 mW/cm^2; contact times, ≥5 h), all MS2 was inactivated. These findings may lead to development of a standard method to test decontamination of respirators challenged by viral droplets.

During an infectious disease outbreak widespread panic can result from a limited understanding of the transmission route. Although some research points to a larger role for droplet nuclei (21, 28), other research suggests that droplets are the principal means of transmitting respiratory infections (10, 26). Droplets containing an infectious microorganism are believed to be transmitted to individuals who directly inhale the droplets resulting from coughing by carriers in close proximity or who ingest droplets spread to the mouth or the nose via the hands (7, 15). Large droplets were first defined as droplets more than 100 μm in diameter by Wells (30). Elsewhere, however, droplets more than 5 μm (23) or 10 μm (8) in diameter are often treated as large droplets. In this paper, the term "viral droplet" refers to all virus-containing liquid particles that retain their original size without significant evaporation, regardless of their specific size.

N95 filtering facepiece respirators (FFRs) are routinely employed to prevent exposure of workers to biological hazards such as severe acute respiratory syndrome, tuberculosis, and novel H1N1 influenza A (6). In addition to CDC interim guidance (6), a recent report from the National Academies' Institute of Medicine suggests that healthcare workers who are in close contact with individuals with novel H1N1 influenza illnesses should use fit-tested N95 respirators to reduce the risk of infection (18). This report also recommends increased research on influenza transmission and respiratory protection, which would enable policy makers to update these types of recommendations as additional disease prevention data become available.

Current best practices suggest that once an FFR is worn in the presence of an infected patient, it should be considered potentially contaminated and discarded (5). However, during a pandemic outbreak a shortage of FFRs could occur (4). According to another report from the Institute of Medicine, during a 42-day influenza pandemic outbreak over 90 million N95 FFRs will be needed to protect workers in the healthcare sector (4). Furthermore, this report suggested that FFR reuse following decontamination should be considered a possible solution to deal with anticipated FFR shortages. Low-temperature biological decontamination methods have been suggested as a possible solution, but additional research needs to be done to determine whether infectious organisms can survive the decontamination process and if the decontamination method changes respirator fit (29).

While it is well known that droplets play a role in the transmission of some respiratory infections, there is a lack of knowledge and data on the effectiveness of decontamination methods applied to respirators and porous personal protective equipment. There are several test methods for evaluation of the effectiveness of decontamination procedures for liquids and for hard porous or nonporous surfaces when they are challenged with viruses (2, 3, 27), while other methods are used to assess the effectiveness of decontamination procedures for

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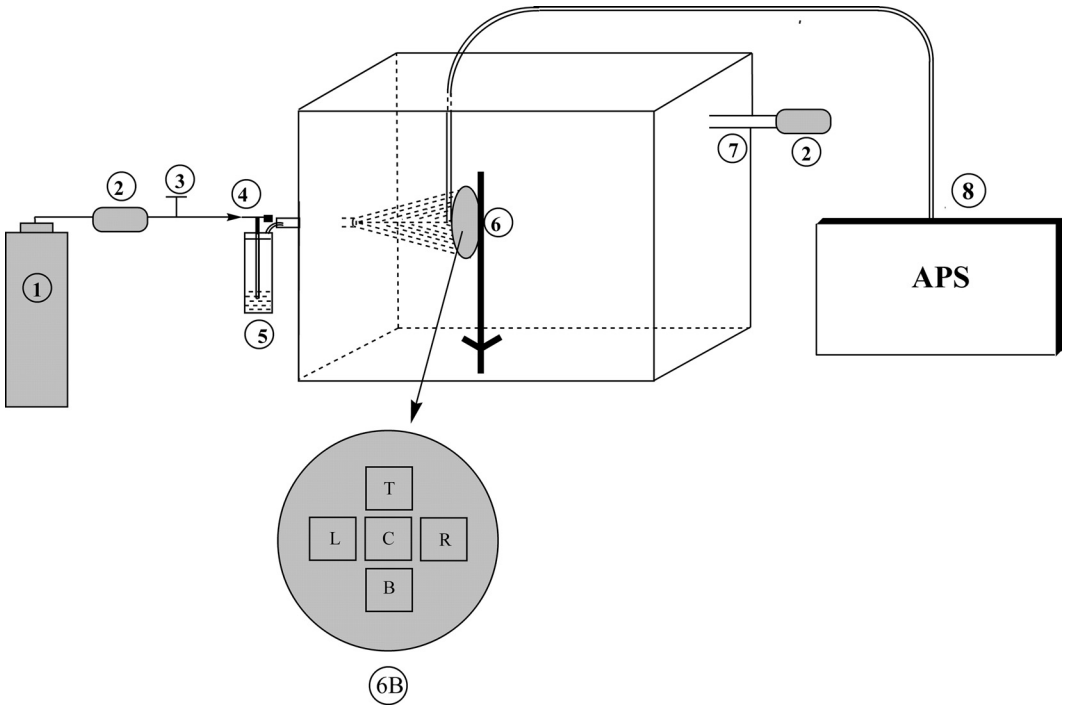

FIG. 1. Schematic diagram of the droplet chamber test system. 1, compressed air supply; 2, HEPA filter; 3, airflow regulator; 4, nebulizer air inlet; 5, nebulizer; 6, test FFR; 6B, sample coupons on the respirator (T, top; C, center; B, bottom, L, left; R, right); 7, exhaust port with HEPA filter; 8, APS.

FFRs when they are challenged with viral droplet nuclei (12). However, there is no test method to evaluate the effectiveness of biological decontamination procedures for disposable FFRs after they are challenged with viral droplets (liquid droplets containing a virus) whose sizes are similar to the sizes of droplets expelled by humans.

Therefore, the aim of the present study was to develop a test system to evaluate the effectiveness of procedures for decontamination of respirators contaminated with viral droplets. The system was validated using two possible decontamination strategies: sodium hypochlorite and UV irradiation. It was hypothesized that the more potent decontamination treatments would result in corresponding larger decreases in the number of viable viruses recovered from the respirators than the less aggressive treatments.

MATERIALS AND METHODS

Preparation of MS2 virus, plaque assay, and droplet test solution. (i) Preparation of MS2 virus. Tryptone yeast extract glucose broth 271 was prepared using the method of the American Type Culture Collection (ATCC) (www.atcc.org). This culture medium was designated 271B and used for growth of *Escherichia coli*, storage of MS2, a plaque assay, and the MS2 extraction and recovery process. These procedures have been used previously in our lab (12).

E. coli ATCC 15597 and bacteriophage MS2 (ATCC 15597-B1) were obtained from the ATCC. MS2 virus was replicated using *E. coli* as the host. 271B was inoculated with an aliquot of frozen *E. coli* (20 μl of *E. coli* in 10 ml of 271B) and incubated overnight at 37°C. Fresh 271B (100 ml) was inoculated with 1 ml of the overnight culture of *E. coli* and then incubated at 37°C with shaking at 100 rpm for 3 to 4 h. An MS2 stock (1.5 ml; ATCC 15597-B1) was added to the overnight culture of *E. coli* at a concentration of 10^9 PFU/ml and incubated with shaking overnight at 37°C (multiplicity of infection, ~20). Lysozyme (0.05 mg/ml) was added to the MS2 overnight culture to liberate the MS2, and then the mixture was shaken vigorously for 5 to 10 min. The lysis solution containing MS2 was then centrifuged at 7,100 × *g* for 30 min at 4°C (IEC Multi RF; Thermo Electron Corporation), and the supernatant containing MS2 was filtered through a sterile 0.22-μm-pore-size filter (Whatman International Ltd., Maidstone, United Kingdom) into a sterile container. MS2 coliphage were enumerated using a standard assay method (1) as described below, and the final MS2 suspension (10^{11} PFU/ml) was stored at 4°C and used within 1 month after production. This MS2 suspension was designated a stock MS2 suspension. MS2 was selected for the study based on its moderate resistance to decontamination, survivability, ease of preparation and assay, and nonpathogenicity (19, 31).

(ii) Plaque assay. A standard overlay agar assay method was used to enumerate the viruses (1). Sterile glass tubes containing 100 μl of the overnight bacterial host (*E. coli*) and 100 μl of the diluted MS2 phage were warmed in a water bath at 45°C. Three milliliters of melted soft agar (0.5% agar) was added to each tube and mixed thoroughly. The mixture was then poured into a labeled petri dish containing hard agar (1.5% agar). The dishes were covered, and the agar was allowed to gel. The plates were then inverted and incubated at 37°C overnight. The plaques were counted, multiplied by the dilution factor, and divided by the sample volume (in milliliters) to obtain the titer expressed in PFU/ml.

(iii) Droplet test solution. 271B was used as a droplet test solution (nebulizer fluid). All MS2 droplet test solutions were prepared by diluting the stock MS2 suspension in 271B to obtain a final concentration of approximately 10^7 PFU/ml. This MS2 concentration was chosen to ensure a loading level of $\geq 1 \times 10^3$ PFU/ml so that there was an adequate detection limit for the bioassay described below.

DPARTS. (i) Droplet test system. Viral droplet size is affected by atmospheric humidity. To generate droplets from which there was not significant evaporation that decreased their original size, a droplet-phase aerosol respirator test system (DPARTS) was designed and constructed (Fig. 1) so that the relative humidity (RH) in it was higher than that of the surrounding air. The DPARTS used for loading the FFRs with MS2 droplets consisted of a compressed air supply, high-efficiency particulate air (HEPA) filters, an airflow regulator (Ashcroft, Costa Mesa, CA), a six-jet Collison nebulizer (BGI, Inc., Waltham, MA) with a short tube (length, 3 cm; diameter, 1.5 cm) connecting the nebulizer outlet to the wall of an exposure chamber, a 43-liter exposure chamber (acrylic chamber with a hinged front door; Vandiver Enterprises, Zelienople, PA), a test respirator holder containing an FFR, an exhaust port, and an aerodynamic particle sizer

(APS) (model 3321; TSI Inc., Shoreview, MN) with a full-size distribution range of 0.5 to 20 μm.

The exposure chamber (35 by 35 by 35 cm) had an internal volume of approximately 43 liters. An airflow regulator was used to control the air pressure in the nebulizer at 20 lb/in^2 and produced an airflow rate of approximately 12 liters/min. The chamber was maintained at a slightly positive pressure (less than 0.1-in. water column pressure) to ensure that particles did not leak into the chamber. The exhaust port (diameter, 2.5 cm) was left in the open position to remove excess air during droplet sampling. An FFR mounted in the respirator holder was located 15 cm from the droplet outlet inside the chamber. MS2 coliphage was suspended in 271B, and MS2 droplets were generated using the six-jet Collison nebulizer. The chamber was also equipped with a 0.8-cm-diameter port for droplet sampling with the APS.

(ii) Characterization of MS2 droplets in the DPARTS. (a) Size distribution of MS2 droplets. The concentration and size distribution of the MS2 droplets were measured in the front center area of a respirator using an APS with a probe that was 15 cm from the droplet outlet inside the chamber (Fig. 1). The probe connected to the APS was used only in these size distribution experiments and in the experiments described below to determine the uniformity of the aerosol concentrations in the locations used for loading. The APS measured airborne droplet sizes ranging from 0.5 to 15 μm. A TESTO 635-1 humidity- and temperature-measuring instrument was utilized to measure the RH and temperature in the exposure chamber.

(b) Characterization of uniform loading of MS2 droplets. To further investigate the concentrations and size distributions of MS2 droplets, the APS was used to analyze the viral droplet data obtained at different respirator locations (top, center, bottom, left, and right areas of respirator samples) (Fig. 1).

N95 test respirator and viral droplet loading onto respirators. (i) N95 respirator. The N95 FFR (model N1105; Willson, Santa Ana, CA) used in this study is a National Institute for Occupational Safety and Health (NIOSH)-approved FFR that can be used by healthcare workers for protection against particulate hazards. This FFR model is comprised of three layers. The outermost and innermost layers are made from hydrophilic materials. The hydrophobic middle layer is composed of melt-blown polypropylene fibers with an electrical charge designed to enhance the efficiency of capture of submicron particles.

(ii) Viral droplet loading onto respirators. During development of the experimental procedure for loading, the need for sufficient viral droplet particles was considered in order to establish appropriate loading levels to permit adequate detection. Test bioassay samples were diluted appropriately and plated so that there were 30 to 300 PFU/plate to ensure acceptable data quality (1). The number of PFU/ml was determined by multiplying the average number of countable plaques (30 to 300 PFU) by the dilution factor. Based on the data quality objective of the bioassay technique and the decontamination method (as described below for decontamination experiments), the minimum detection limit for the loading level had to be at least 1×10^3 PFU/ml for an adequate detection limit.

An FFR mounted in the respirator holder was located 15 cm from the droplet outlet inside the DPARTS chamber (Fig. 1). All MS2 nebulizer samples were prepared by diluting the stock MS2 suspension with 271B to obtain a final concentration of approximately 10^7 PFU/ml. Each MS2 solution (45 ml) was added to the nebulizer glass jar for loading. After the chamber was sealed (with the exception of the exhaust port, which was in the open position during loading), the compressed air valve was opened (rate of nebulizer airflow, 12 liters/min; 20 lb/in^2), and the air passed through the nebulizer to generate MS2 droplets (the rate for the volumetric MS2 suspension leaving the nebulizer was approximately 0.22 ml/min) and into the exposure chamber for subsequent loading onto the FFR. A stopwatch was used to measure the duration of loading. MS2 droplets were generated continuously, and the RH in the area of the respirator holder containing the FFR was monitored. After 10 s, the RH reached 95% and then stabilized at 95 to 99% for the duration of the exposure period (a high RH was used to maintain droplet particle size without significant evaporation; it was not intended to reflect real workplace conditions). Once the MS2 droplet load reached the desired value (loading time, 5 min; desired load = [MS2 nebulizer suspension concentration in PFU/ml] × [rate of MS2 suspension leaving the nebulizer in ml/min] × [loading time in min] = 10^7 PFU/ml × 0.22 ml/min × 5 min = approximately 10^7 PFU), the airflow was stopped. Then the exposed FFR was retrieved and saved for use in decontamination experiments.

Decontamination experiments. (i) Sodium hypochlorite decontamination experiment. (a) Sodium hypochlorite. Sodium hypochlorite (NaOCl) solutions (stock solution, 6% NaOCl), commonly known as bleach, were used for chemical decontamination. The stock sodium hypochlorite solution (Clorox regular bleach; Environmental Protection Agency registration no. 5813-50) was obtained from a commercial supplier. In the chemical decontamination experiments, all sodium hypochlorite working solutions (0.005, 0.01, 0.05, 0.1, 0.25, 0.5, and 0.75% sodium hypochlorite) were freshly prepared by diluting the 6% sodium hypochlorite stock solution with purified water; these solutions contained 0.06, 0.11, 0.55, 1.10, 2.75, 5.50, and 8.25 mg/liter of sodium hypochlorite, respectively, and were mixed gently at room temperature with continuous stirring for 15 min.

Each FFR loaded with MS2 was submerged in 1 liter of a sodium hypochlorite solution or purified water. Treatment with water (with no NaOCl) was used as a baseline treatment to determine losses due to handling of FFR samples during the chemical decontamination process. In the chemical decontamination experiments control samples of FFRs loaded with MS2 were not submerged in either purified water or a sodium hypochlorite solution. If the efficiency of viral droplet loading onto FFRs is 100%, the number of viruses recovered from the controls and the number of viruses loaded into droplets are the same. For the sodium hypochlorite decontamination experiments, both sides of a complete FFR were decontaminated by submerging the FFR in a sodium hypochlorite solution. After 10 min of treatment, the respirator was removed from the purified water or sodium hypochlorite solution and air dried for 2 min. A toxicity control to determine if there was any interference by residual sodium hypochlorite with the chemical inactivation process under these conditions (10 min of sodium hypochlorite treatment and 2 min of air drying) was examined in a previous study (12). The results showed that no residual sodium hypochlorite interfered with subsequent bioassays (12). Three replicate tests ($n = 3$) were carried out for each sodium hypochlorite concentration. Each respirator was cut into coupons (2 cm by 2 cm), and each coupon was then placed in 10 ml of 271B in a 50-ml conical tube for extraction.

(b) Virus recovery. MS2 was extracted from the coupons by vortexing them for 2 min. When extraction procedure was complete, the coupons were discarded, and the supernatant was assayed for viable MS2 as previously described.

(c) Efficacy of the sodium hypochlorite decontamination. The number of viable MS2 phage was determined by a plaque assay. The efficacy of decontamination (ED) for MS2 was calculated by determining the log reduction as follows: ED = log ($N°/N$), where $N°$ is the mean number of viable MS2 phage applied to the control coupons (i.e., coupons not subjected to decontamination) and N is the number of viable MS2 phage recovered from test coupons after decontamination.

(ii) UV decontamination experiment. (a) UV decontamination procedures. UV decontamination was carried out using a UV germicidal lamp in a biological safety cabinet (SterilGARD III model SG403A; Baker Company, Sanford, ME). A low-pressure mercury arc lamp (5.5 mg Hg; lamp type, TUV 36TS 4P SE; lamp voltage, 94 V; lamp wattage, 40 W; wavelength, 253.7 nm) was used as the UV source. The UV intensity on the sample surface was measured using a UVX-25 digital radiometer (model E28457; Cole-Parmer, Vernon Hills, IL). After exposure to MS2, an FFR was treated with UV irradiation using a UV source intensity of 0.4 mW/cm^2 at the FFR surface (distance from lamp to sample surface, 42 cm). The UV doses (J/cm^2) used in the decontamination treatments were calculated by multiplying the average UV intensity at the FFR surface by the irradiation time (in seconds). In these experiments, the UV treatment was applied only to the side of the FFR closest to the nebulizer (Fig. 1). To eliminate the possibility that MS2 contaminated the inside surface of the FFR by traveling directly from the back of the FFR holder during the droplet loading step, a second FFR was placed behind the FFR sample being tested to act as a protective cover.

Because of the relatively low intensity of the UV that reached the FFR, long irradiation times (1, 2, 3, 4, and 5 h; control, 0 h) were used to obtain appropriate UV doses. The survival of the MS2 virus on the FFRs at different times was also examined by storing the MS2-contaminated FFR samples for 1, 2, 3, 4, and 5 h (at the same temperature and RH) but without UV irradiation (control experiments). Three replicate tests were carried out for each irradiation time and each control experiment. To ensure that the temperature and RH did not adversely affect the efficacy of UV decontamination for inactivation of MS2 virus (20), all UV decontamination and control experiments were carried out at 25 ± 1°C and 53% ± 1% RH. After the decontamination and control treatments, each FFR was cut into square coupons (2 cm by 2 cm), and each coupon was placed in 10 ml of 271B in a 50-ml conical tube for extraction.

(b) Virus recovery. MS2 recovery was determined as described above for the sodium hypochlorite decontamination experiment.

(c) Efficacy of UV decontamination. The efficacy of UV decontamination for viable MS2 was calculated as described above for the efficacy of sodium hypochlorite decontamination.

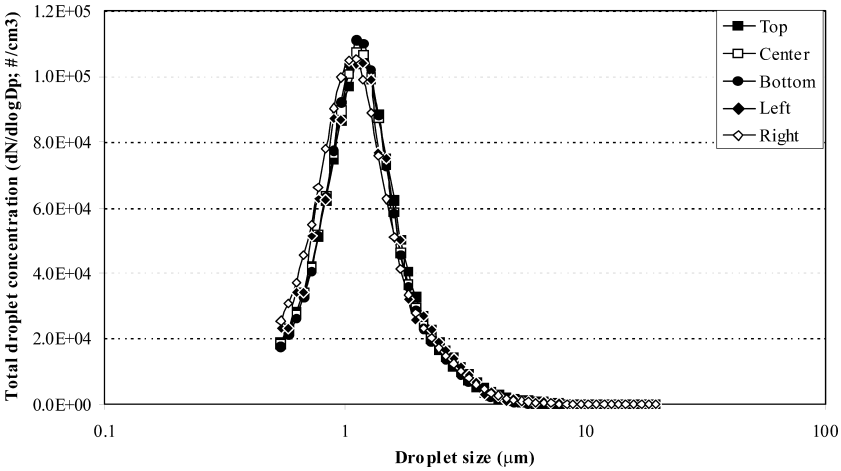

FIG. 2. Size distribution of droplets containing MS2 virus at different respirator locations.

RESULTS

Characterization of viral droplets in the DPARTS. The DPARTS was constructed to generate droplets containing MS2 virus and to load them onto FFRs. The RH and temperature in the areas of the respirator holder containing an FFR were maintained at 95 to 99% and 22 ± 3°C, respectively. Under these conditions, droplets containing MS2 virus generated in the exposure chamber retained their size without significant evaporation that decreased their original size.

Size distributions, which indicate the concentration of droplets as a function of droplet diameter, are shown in Fig. 2. The size range of the droplets loaded directly onto an FFR was measured in the front center area of the respirator and was found to be 0.5 to 15 μm, and the majority of the droplets were in the size range from 0.74 to 3.5 μm. Experimental distribution results for the viral droplets showed that the count mean diameter and the mass mean diameter measured by the APS in the specific range from 0.5 to 15 μm were 1.35 μm and 3.28 μm, respectively.

The average concentration of droplets (total number of droplets per cm^3) as a function of particle size at different respirator locations (top, center, bottom, left, and right areas) was also characterized. Comparison of the results indicated that the droplet concentrations and the droplet size distributions at different respirator locations were similar (Fig. 2). The average concentrations of droplets were found to be 8.5×10^3, 8.7×10^3, 8.3×10^3, 8.5×10^3, and 8.5×10^3 droplets/cm^3 ($n = 3$) for the top, center, bottom, left, and right areas of respirators, respectively. These results showed that the droplet test system not only generated a designed droplet size but also generated similar droplet particle counts for different respirator areas.

Sodium hypochlorite decontamination experiment. The results of our previous study to investigate the filtration performance of FFRs before and after treatment with sodium hypochlorite showed that there was no significant increase in particle penetration levels (29). In other words, hypochlorite treatment of an FFR had no deleterious effect on performance. Table 1 shows a statistical summary of the results for each sodium hypochlorite dose (mg/liter), including the negative control for each test and the control for each sodium hypochlorite dose. The efficiency of sodium hypochlorite decontamination of FFR samples loaded with MS2 is also shown in Table 1. As expected, higher sodium hypochlorite doses resulted in greater log reductions in the number of MS2 coliphage. Generally, 10 min of decontamination with all solutions with sodium hypochlorite concentrations of ≥2.75 mg/liter resulted in >3-log reductions in the MS2 coliphage level

TABLE 1. Log_{10} reductions in the MS2 level with different sodium hypochlorite doses

Solution	Composition	NaOCl dose (mg/liter)	Avg amt of MS2 recovered (log)	Efficacy of decontamination
Control		0	7.29	ND[a]
No NaOCl	100% H_2O	0	6.92	0.37 ± 0.67
0.005% NaOCl	0.005% NaOCl, 0.08% other ingredients, 99.92% H_2O	0.06	6.63	0.66 ± 0.47
0.01% NaOCl	0.01% NaOCl, 0.16% other ingredients, 99.83% H_2O	0.11	6.56	0.73 ± 0.42
0.05% NaOCl	0.05% NaOCl, 0.8% other ingredients, 99.15% H_2O	0.55	5.94	1.35 ± 0.66
0.1% NaOCl	0.1% NaOCl, 1.6% other ingredients, 98.3% H_2O	1.10	5.29	2.00 ± 0.48
0.25% NaOCl	0.25% NaOCl, 4% other ingredients, 95.75% H_2O	2.75	4.16	3.13 ± 1.30
0.5% NaOCl	0.5% NaOCl, 8% other ingredients, 91.5% H_2O	5.50	2.92	4.37 ± 0.40
0.75% NaOCl	0.75% NaOCl, 11.75% other ingredients, 87.5% H_2O	8.25	BMDL[b]	UN[c]

[a] ND, not determined.
[b] BMDL, below the minimum detection limit.
[c] UN, undefined.

TABLE 2. Log_{10} reductions in the MS2 level with different UV doses

UV decontamination time (h)	Distance from lamp to FFR surface (cm)	UV intensity on FFR surface (mW/cm^2)	UV dose (J/cm^2)	Avg amt of MS2 recovered (log)	Efficacy of decontamination
Control			0	6.76	ND[a]
1	42	0.4	1.44	4.93	1.83 ± 0.49
2	42	0.4	2.88	4.11	2.64 ± 0.60
3	42	0.4	4.32	3.75	3.00 ± 0.61
4	42	0.4	5.76	3.59	3.16 ± 0.49
5	42	0.4	7.20	BMDL[b]	UN[c]

[a] ND, not determined.
[b] BMDL, below the minimum detection limit.
[c] UN, undefined.

(decontamination efficiency, >99.9%; relative standard deviation [RSD], <41.5%). An approximately 4-log reduction in the MS2 coliphage level was obtained with 5.5 mg/liter and 10 min of treatment (RSD, <9.2%). No detectable MS2 was found after treatment with a sodium hypochlorite dose of ≥8.25 mg/liter for 10 min.

UV decontamination experiment. In a control experiment, the survival of MS2 virus on FFR samples without UV irradiation was determined and was found to be 5.4×10^5, 5.5×10^5, 5.1×10^5, 5.0×10^5, and 4.9×10^5 PFU/ml at 1, 2, 3, 4, and 5 h, respectively. Comparison of these results indicated that ~90% of the viruses on these FFR samples not exposed to UV were still viable even after 5 h of storage compared to the 1-h control. A statistical summary of the UV decontamination data is shown in Table 2. The UV doses applied (the product of the average UV intensity at the respirator sample and the irradiation time) are also included in Table 2. Higher UV doses resulted in greater reductions in MS2 coliphage levels (Table 2). Generally, an approximately 3-log reduction in the MS2 coliphage level was obtained with the 4.32-J/cm^2 dose (RSD, <20.3%; decontamination efficiency, >99%). No MS2 viruses were detected with UV irradiation doses of ≥7.20 J/cm^2 (Table 2).

DISCUSSION

The experimental results for droplet size distribution shown here demonstrated that the sizes of the MS2 droplets ranged from 0.5 to 15 μm and that the sizes of the majority of the particles ranged from 0.74 to 3.5 μm. The results also indicated that with the high concentration of solutes present in the virus propagation medium and with the small MS2 virion size (diameter, 27.5 nm) (14), addition of MS2 virus to the medium had little effect on the droplet size distribution. In general, the size distribution of viral droplets is controlled by the properties of the nebulizer liquid medium and the nebulizer-generator method, not by the physical size of the viruses themselves (12, 17). MS2 droplet loading on different areas of the FFR was investigated. The average concentrations of droplets at different respirator locations (top, center, bottom, left, and right regions) were not significantly different (8.3×10^3 to 8.7×10^3 droplets/cm^3). These results also showed that the droplet test system not only generated a designed droplet size but also loaded the droplets at different locations on the respirator uniformly.

The role of droplet particle size on the efficacy of decontamination of virus-containing droplets deposited on FFRs is unknown. Although several studies have reported the sizes and numbers of particles (droplets and/or droplet nuclei) emitted by healthy and symptomatic human test subjects while they are sneezing, coughing, talking, and breathing (9, 11, 13, 16, 22, 24, 32), there is no consensus concerning the typical droplet size or the suspension medium that should be used in development of a test method. Thus, in this study we targeted a particle size range that could be readily and reproducibly obtained using simple off-the-shelf components and is also found for the particles emitted by human subject in studies. The Collison nebulizer used in this study is limited to particles less than a few micrometers in diameter, but in our lab it has proven to be quite reliable and fairly inexpensive. In a study which obtained data specifically for droplets, Yang and coworkers observed coughed droplets with sizes ranging from 0.62 to 13.9 μm (32), and the average size was 8.35 μm. Although the sizes of the droplets generated by the DPARTS (Fig. 2) are slightly smaller (count mean diameter, 1.35 μm; mass mean diameter, 3.28 μm), we felt that the DPARTS offers a reasonable compromise. Thus, the viral droplets generated by the DPARTS (diameters, 0.74 to 3.5 μm) are in a size range that could possibly contaminate an FFR.

The humidity in the area of the respirator holder containing the FFR was kept at 95 to 99% RH to help maintain the original sizes of the viral droplets. If drier air were used, the moisture in the droplets would rapidly evaporate to form droplet nuclei. The effectiveness of biological decontamination methods for droplet nuclei containing viruses is the subject of other work in our lab (12). In this study, the droplets were not allowed to evaporate until they were deposited on the FFR. Previous research suggested that droplet and droplet nuclei interact differently with FFRs (25), and thus the development of separate test methods is useful for understanding the differences in these interactions.

As expected, treatment with sodium hypochlorite at high concentrations was found to be an efficient chemical decontamination method for MS2 virus. The efficacy of chemical decontamination by sodium hypochlorite was a function of concentration. Sodium hypochlorite doses of 2.75 to 5.50 mg/liter resulted in approximately 3- to 4-log reductions in the MS2 coliphage level, while higher sodium hypochlorite doses resulted in even greater log reductions in the MS2 coliphage level. No survival of MS2 was observed with a sodium hypochlorite dose of ≥8.25 mg/liter for 10 min.

The relative levels of survival of MS2 virus on the FFR

control samples (i.e., samples subjected to similar environmental conditions and storage times but without UV irradiation) were found to be similar for the different storage times. Because ~90% of the viruses were viable after 5 h, the effect of the storage parameters was negligible compared to the data for the numbers of viable virus recovered from FFR samples after UV decontamination. Thus, the calculated values for the efficiency of decontamination shown in Table 2 are primarily due to UV exposure. In UV irradiation decontamination experiments, an approximately 3-log reduction in the level of MS2 coliphage was observed with a dose of 4.32 J/cm^2. UV irradiation resulted in radical formation due to the interaction of UV light and the nebulizer medium containing MS2 virus. As expected, the efficacy of physical decontamination for the various doses of UV irradiation was a function of the UV dose. The UV decontamination treatment was more efficient when the UV irradiation dose was higher due to the larger numbers of radicals generated. Since it did not leave any odor or toxicity in the FFRs, the UV irradiation method should be considered for future research on respirator decontamination. For example, additional research is needed to determine what effects (if any) the presences of pleats or folds on an FFR have on the efficacy of UV decontamination.

Study limitations. The results showed that the DPARTS was able to generate and load viral droplets onto FFRs uniformly, which allowed us to evaluate the effectiveness of procedures for decontamination of respirators successfully. However, the findings are limited, and the data presented here are applicable only to the relative decontamination efficiencies of sodium hypochlorite and UV irradiation with droplets containing nonenveloped MS2 virus on a single FFR model; other known pathogenic respiratory enveloped viruses may not be behave similarly. The levels of decontamination obtained with sodium hypochlorite and UV in this study cannot be used directly to estimate the efficacies of decontamination for other viruses with different genes (RNA or DNA) or different protein coats. Additional studies examining these topics are needed.

It must also be noted that in this study we targeted a droplet size range that could be readily and reproducibly obtained using our experimental setup. Thus, the composition and size of droplet particles in this study may not mimic exactly the composition and size of droplet particles from human respiratory secretions. Future studies should look for new suspension media and other methods for generating particles to better approximate the composition and size of droplet particles from human respiratory secretions and to determine if larger droplets can also be generated reproducibly.

The data presented in this paper are applicable only to FFRs containing a hydrophilic outer layer; other FFRs with only hydrophobic materials may behave differently. These studies should be repeated with other types of FFRs that contain a hydrophobic outer layer. Studies are also needed to compare the efficacies of decontamination for viruses applied to respirators using different deposition methods, including spiking (liquid deposition) and a bioaerosol respirator test system (droplet nuclei) (12), as well as the DPARTS.

Conclusions. The DPARTS was constructed to generate representative MS2 virus-containing droplets and to load them onto FFRs uniformly. Droplet particles containing MS2 in the areas of the respirator holder containing an FFR (with 95 to 99% RH) maintained their sizes without significant evaporation. The results demonstrated that the size range of the droplets was 0.5 to 15 μm and that the majority of the droplet particles were between 0.74 and 3.5 μm in diameter. Treatment with sodium hypochlorite (bleach) was an efficient chemical decontamination method for MS2 virus loaded onto FFRs. Treatment with low sodium hypochlorite doses (2.75 to 5.50 mg/liter) resulted in approximately 3- to 4-log reductions in the levels of MS2 coliphage, while treatment with higher sodium hypochlorite doses (≥8.25 mg/liter) resulted in no detectable MS2 virus. UV irradiation was also demonstrated to be an efficient physical decontamination treatment for MS2 virus loaded onto FFRs. Treatment with low UV irradiation doses (4.32 to 5.76 J/cm^2) resulted in 3.00- to 3.16-log reductions in the levels of MS2 coliphage, while treatment with higher UV irradiation doses (≥7.20 J/cm^2) resulted in no detectable MS2 virus.

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